Plasma 25-Hydroxyvitamin D and Risk of Non-Melanoma and Melanoma Skin Cancer: A Prospective Cohort Study

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Sun exposure is a major risk factor for skin cancer and is also an important source of vitamin D. We tested the hypothesis that elevated plasma 25-hydroxyvitamin D (25-OH-vitD) associates with increased risk of non-melanoma and melanoma skin cancer in the general population. We measured plasma 25-OH-vitD in 10,060 white individuals from the Danish general population. During 28 years of follow-up, 590 individuals developed non-melanoma skin cancer and 78 developed melanoma skin cancer. Increasing 25-OH-vitD levels, by clinical categories or by seasonally adjusted tertiles, were associated with increasing cumulative incidence of non-melanoma skin cancer (trend $P=2\times10^{-15}$ and $P=3\times10^{-17}$) and melanoma skin cancer ($P=0.003$ and $P=0.001$). Multivariable adjusted hazard ratios of non-melanoma skin cancer were 5.04 (95% confidence interval (CI): 2.78–9.16) for 25-OH-vitD $\geqslant$50 vs. $<$25 nmol l^{-1}, and 4.02 (2.45–6.60) for top versus bottom tertile. Multivariable adjusted hazard ratios of melanoma skin cancer were 4.7 (0.96–23.3) for 25-OH-vitD $\geqslant$50 vs. $<$25 nmol l^{-1}, and 6.3 (1.38–28.8) for top versus bottom tertile. The absolute 20-year risk was 11% for non-melanoma skin cancer and 1.5% for melanoma skin cancer, in participants with age $>$60 years, 25-OH-vitD winter levels $\geqslant$50 nmol l^{-1}, and performing outdoor exercise. In conclusion, we show that increasing levels of 25-OH-vitD are associated with increased risk of non-melanoma and melanoma skin cancer.

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INTRODUCTION

UV radiation from sun exposure is a main environmental risk factor for non-melanoma and melanoma skin cancer (Armstrong and Kricker, 1993, 2001; Kricker *et al.*, 1995; Gallagher *et al.*, 1995a, b). However, the wavelength of UV radiation that causes DNA damage in skin cells also induces vitamin D production in keratinocytes (Holick *et al.*, 1977, 1979; Adams *et al.*, 1982; MacLaughlin *et al.*, 1982; Freeman *et al.*, 1989), which promotes differentiation and reduces proliferation in normal skin cells and skin cancer cell lines (Colston *et al.*, 1981; Eisman *et al.*, 1987; Evans *et al.*, 1996; Bikle, 2004; Tang *et al.*, 2011b). Furthermore, vitamin D receptor knockout mice have increased susceptibility toward UV radiation–induced skin tumors (Zinser *et al.*, 2002).

Thus, an increase in vitamin D levels, measured by plasma 25-hydroxyvitamin D (25-OH-vitD), might reduce the risk of non-melanoma and melanoma skin cancer due to the aforementioned effects of vitamin D (Tang *et al.*, 2010; Newton-Bishop *et al.*, 2011). In contrast, if plasma 25-OH-vitD is a surrogate marker for sun exposure then higher levels may be associated with increased rather than decreased risk of non-melanoma and melanoma skin cancer (Asgari *et al.*, 2010).

We tested the hypothesis that elevated plasma 25-OH-vitD levels associate with increased risk of non-melanoma and melanoma skin cancer in the general population. For this purpose, we studied 10,060 white individuals from the Copenhagen City Heart Study followed up for up to 28 years. Two aspects make this cohort unique: in Northern Europe, UV-B radiation from the sun is only adequate for sufficient endogenous vitamin D production in the skin during the summer months from May to September, and food has never been fortified with vitamin D in Denmark. Thus, this cohort from the Danish general population allows the determination of the natural history of the association of vitamin D levels with risk of non-melanoma and melanoma skin cancer. However, our data do not account for sun exposure and sunburns during holidays in Denmark or abroad.

RESULTS

The seasonal variation in plasma 25-OH-vitD is depicted in Figure 1, with the highest levels in September and the lowest levels in February. Furthermore, we remeasured plasma 25-OH-vitD levels in 400 healthy participants of the 10,060

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Abbreviations: BMI, body mass index; CI, confidence interval; 25-OH-vitD, 25-hydroxyvitamin D

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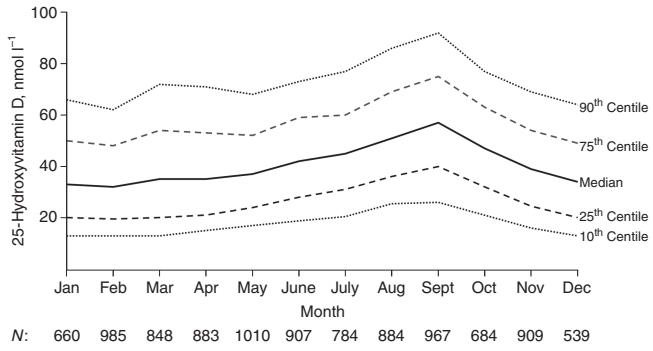

Figure 1. Seasonal variation in 25-hydroxyvitamin D. Seasonal variation in 25-hydroxyvitamin D levels according to calendar month of blood sampling (based on 10,060 individuals from the Danish general population, the Copenhagen City Heart Study). Apr, April; Aug, August; Dec, December; Feb, February; Jan, January; Mar, March; Nov, November; Oct, October; Sept, September.

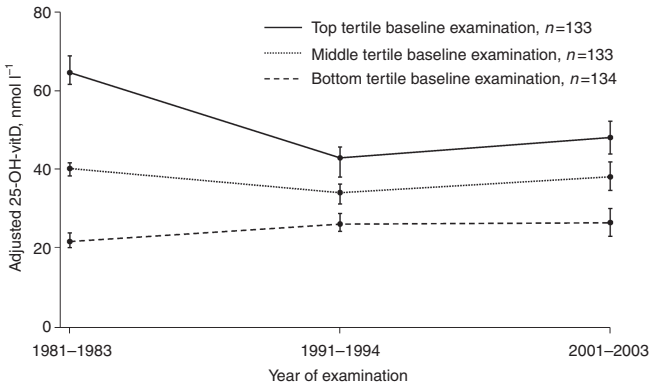

Figure 2. Repeated measurements of plasma 25-hydroxyvitamin D (P-25-OH-vitD) 10 and 20 years apart. Median adjusted P-25-OH-vitD levels according to baseline tertiles constructed on the basis of measurements in the 1981–1983 examination. Repeated measurements of P-25-OH-vitD were 10 and 20 years apart on the same individuals. P-25-OH-vitD was adjusted for age, body mass index, and month of blood sampling. Solid circles are median levels and the associated whiskers are 95% confidence intervals (based on 400 of the 10,060 individuals from the Danish general population, the Copenhagen City Heart Study).

participants ~10 and 20 years after the initial measurements: the relationship between baseline, second, and third measurement is illustrated in Figure 2, showing the levels of plasma 25-OH-vitD adjusted for season, age, and body mass index (BMI). There is a narrowing of the range of values in the follow-up measurements, i.e., there is evidence of regression toward the mean. However, the ranking derived from baseline measurements remains throughout the follow-up period, indicating a stable level over longer time periods up to 20 years. Measurements from the 1981–1983 examination correlated with the measurements from the 1991–1994 (Spearman's $\rho = 0.41$, $P = 10^{-17}$) and 2001–2003 examinations (Spearman's $\rho = 0.40$, $P = 10^{-16}$).

Increasing levels of plasma 25-OH-vitD were associated with decreasing age, decreasing cumulative tobacco consumption, decreasing BMI, increasing income, increased intensity of leisure-time activity, and with regular cycling or running (Table 1). Median plasma 25-OH-vitD was 41 nmol l^{-1} for the whole population, 48 nmol l^{-1} among those who later developed non-melanoma skin cancer, and 51 nmol l^{-1} among those who later developed melanoma skin cancer. A total of 590 participants developed non-melanoma skin cancer and 78 participants developed melanoma during up to 28 years of follow-up. Table 1 also shows whether covariates selected *a priori* associate with the risk of skin cancer.

Cumulative incidence

Cumulative incidence of non-melanoma and melanoma skin cancer increased stepwise with increasing levels of plasma 25-OH-vitD, expressed in clinical categories (trend: $P = 2 \times 10^{-15}$ and $P = 0.003$) and in seasonally adjusted percentile categories ($P = 3 \times 10^{-17}$ and $P = 0.001$) (Figure 3).

Relative risk

Adjusted hazard ratios for non-melanoma and melanoma skin cancer increased with increasing levels of plasma 25-OH-vitD, by clinical categories and by seasonally adjusted tertiles, in both of our models (Figure 4). Multivariable adjusted hazard ratios for non-melanoma skin cancer were 5.04 (95% CI: 2.78–9.16) for 25-OH-vitD ⩾50 vs. <25 nmol l^{-1}, and 4.02 (2.45–6.60) for >67th vs. ⩽34th percentile. Multivariable adjusted hazard ratios for melanoma were 4.72 (0.96–23.3) for plasma 25-OH-vitD ⩾50 vs. <25 nmol l^{-1}, and 6.31 (1.38–28.8) for >67th vs. ⩽34th percentile.

Analyzing 25-OH-vitD as a continuous variable showed a multivariable adjusted hazard ratio of 1.23 (1.14–1.32) for non-melanoma skin cancer and 1.45 (1.22–1.73) for melanoma skin cancer per 10 nmol l^{-1} increase in plasma 25-OH-vitD. Similarly, the multivariable adjusted hazard ratio for a 10% increase in seasonally adjusted percentile of plasma 25-OH-vitD was 1.23 (1.15–1.32) for non-melanoma skin cancer and 1.27 (1.05–1.53) for melanoma.

Interaction analyses did not show any significant interactions between 25-OH-vitD, as a continuous variable, and any of the covariates on risk of non-melanoma skin cancer or melanoma.

Relative risk: subgroup analyses

In separate analyses, the association of very high levels of plasma 25-OH-vitD with non-melanoma skin cancer and melanoma were carried out. The multivariable adjusted hazard ratios for non-melanoma skin cancer was 5.28 (95% CI: 1.66–16.8) for 25-OH-vitD ⩾100 vs. <25 nmol l^{-1}. The corresponding multivariable adjusted hazard ratios for melanoma was 9.58 (2.37–38.7).

As melanoma developing in sites often exposed to UV radiation may be different from melanomas in sun-unexposed sites, additional analyses for melanoma risk in sun-exposed sites (head and extremities, 40 cases) versus relatively unexposed sites (trunk and other sites, 38 cases) were carried out. The multivariable adjusted hazard ratio was 1.58 (1.25–2.00) for melanoma in sun-exposed sites per 10 nmol l^{-1} increase in plasma 25-OH-vitD, whereas it was 1.24 (0.93–1.66) for

Table 1. Baseline characteristics according to clinical categories for 25-hydroxyvitamin D plasma levels

	Plasma 25-hydroxyvitamin D (nmol l^{-1})				Non-melanoma skin cancer		Melanoma	
	<25 (*n* = 2,362)	25–49.9 (*n* = 4,035)	>50 (*n* = 3,663)	*P*-value[1]	HR (95% CI)	*P*-value[2]	HR (95% CI)	*P*-value[2]
Men, no. (%)	1,074 (45)	1,760 (44)	1,575 (43)	0.07	1.26 (1.07–1.49)	0.007	1.47 (0.94–2.30)	0.09
Age, years				<0.001		<0.001		0.13
Median	59	59	57					
Interquartile range	50–65	49–65	48–65					
Cumulative tobacco consumption, pack-years				<0.001	1.00 (0.99–1.00)	0.84	0.99 (0.98–1.01)	0.82
Median	24	19	18					
Interquartile range	10–38	5–33	3–31					
Body mass index, kg m^{-2}				<0.001	0.98 (0.96–1.00)	0.04	0.98 (0.93–1.04)	0.60
Median	25	25	24					
Interquartile range	23–29	23–28	22–27					
Income group, no. (%)[3]				<0.001		<0.001		0.55
Low (<84,000 DKr)	927 (40)	1,262 (32)	974 (27)		1		1	
Medium (84,000–192,000 DKr)	1,031 (44)	1,874 (47)	1,714 (47)		1.15 (0.93–1.43)		1.43 (0.77–2.66)	
High (>192,000 DKr)	368 (16)	844 (21)	941 (26)		1.57 (1.23–2.00)		1.28 (0.62–2.65)	
Occupational physical exertion, no. (%)				0.07[4]		0.06[4]		0.35[4]
Low	798 (34)	1,363 (34)	1,273 (35)		1		1	
Occasional	864 (37)	1,596 (40)	1,480 (40)		0.76 (0.63–0.92)		1.06 (0.61–1.84)	
Moderate	510 (22)	858 (21)	752 (21)		0.88 (0.70–1.10)		1.36 (0.75–2.50)	
High	108 (5)	141 (3)	111 (3)		0.68 (0.40–1.16)		1.23 (0.36-4.15)	
Without work	82 (3)	77 (2)	47 (1)		—		—	
Leisure-time activities, hours per week[2]				<0.001		0.08		0.03
⩽2	598 (25)	686 (17)	410 (11)		1		1	
2–4 (light activity)	1,115 (47)	1,986 (49)	1,792 (49)		1.14 (0.88–1.47)		0.89 (0.43–1.81)	
2–4 (heavy activity) or ⩾4 (light activity)	622 (26)	1,282 (32)	1,346 (37)		1.25 (0.96–1.63)		1.61 (0.96–1.63)	
⩾4 (heavy activity)	27 (1)	79 (2)	115 (3)		1.36 (0.75–2.46)		1.87 (0.50–7.03)	
Regular cycling or running				<0.001		<0.001		0.02
No	1,545 (65)	2,274 (56)	1,774 (48)		1		1	
Yes	817 (35)	1,761 (44)	1,889 (52)		1.36 (1.15–1.61)		1.75 (1.10–2.80)	

Abbreviations: CI, confidence interval; HR, hazard ratio.

[1]*P*-values were calculated using Cuzick's non-parametric trend test.

[2]Trend test or use of continuous variable, Cox regression.

[3]1981–1983 income levels (1 US$ ~6 DKr).

[4]Excluding participants not working. The following covariates had missing values: income group: 125 observations, leisure-time activities: 2 observations, and body mass index: 18 observations.

relatively unexposed sites. The corresponding hazard ratios for a 10% increase in seasonally adjusted percentile were 1.31 (1.00–1.71) and 1.19 (0.90–1.57), respectively.

Absolute risk

Absolute 20-year risk of non-melanoma and melanoma skin cancer increased with increasing plasma 25-OH-vitD across age groups and irrespective of whether the blood samples were collected in winter (November–April) or summer (May–October) (Figure 5). The highest absolute 20-year risk of non-melanoma skin cancer of 11% was found in participants aged >60 years with 25-OH-vitD levels ⩾50 nmol l^{-1} in winter months, who regularly performed outdoor exercise. The highest absolute 20-year risk of melanoma of 1.5% was found in participants aged >40 years with 25-OH-vitD levels ⩾50 nmol l^{-1} in winter months, who regularly performed outdoor exercise. Gender was not associated with difference in absolute risk of either of the cancers.

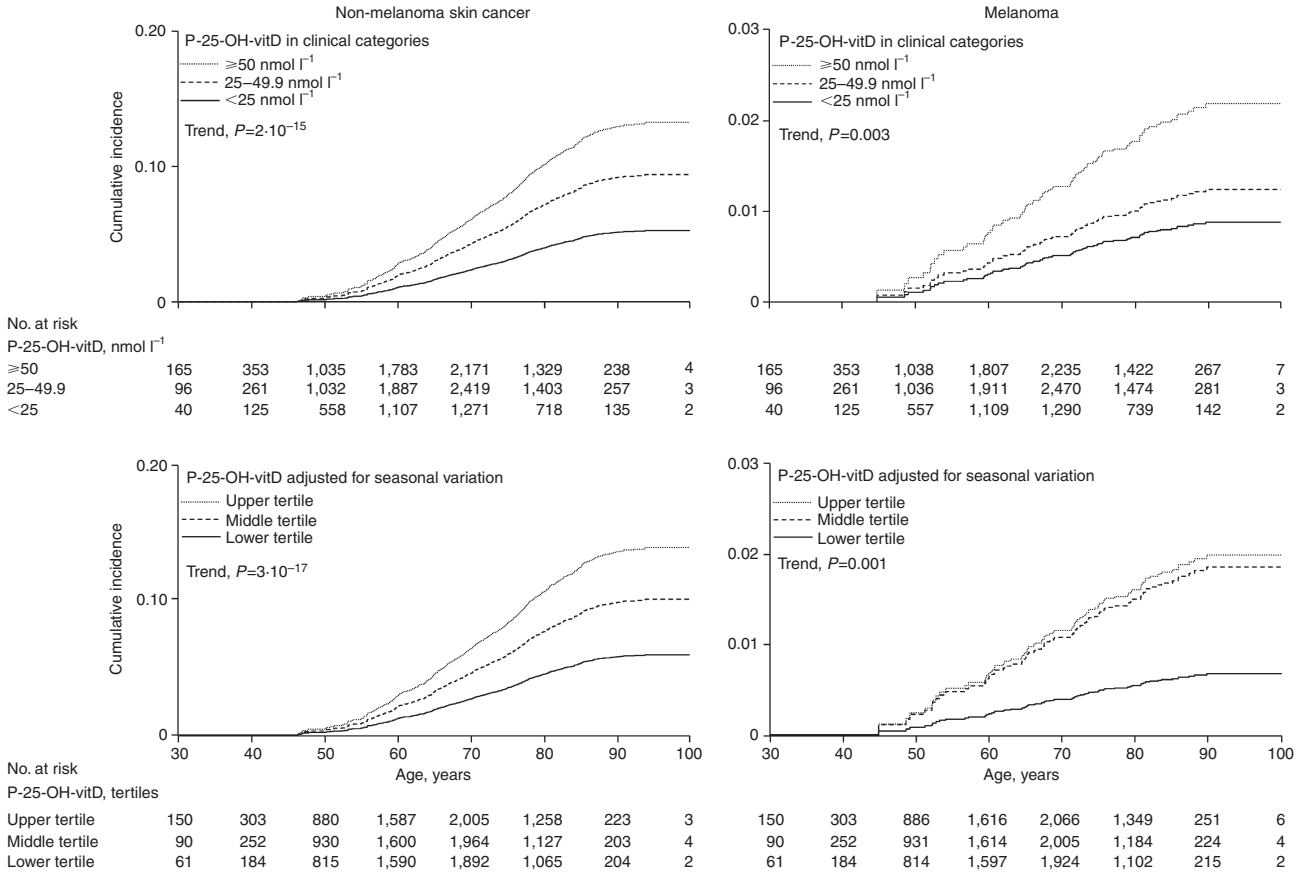

Figure 3. Cumulative incidence of non-melanoma skin cancer and melanoma skin cancer by plasma 25-hydroxyvitamin D (P-25-OH-vitD). Cumulative incidence of non-melanoma skin cancer and melanoma skin cancer by P-25-OH-vitD in clinical categories and in seasonally adjusted tertiles (expressed as percentiles). The cumulative incidences were plotted using Fine and Gray competing risks regression accounting for the competing risk of death. Furthermore, the analysis is adjusted for age and year of birth to account for calendar effects. *P*-values for trend indicate whether increasing levels of 25-OH-vitD are associated with increasing cumulative incidence of non-melanoma skin cancer and melanoma (based on 10,060 individuals from the Danish general population, the Copenhagen City Heart Study, followed for up to 28 years after blood sampling). CI, confidence interval.

DISCUSSION

We found that increasing levels of plasma 25-OH-vitD were associated with increased risk of non-melanoma skin cancer and melanoma in this prospective study of 10,060 participants from the general population followed up for up to 28 years.

Our data lend support to the hypothesis that plasma 25-OH-vitD can be used as a surrogate marker for UV-B radiation from sun exposure and hence be used to identify persons at risk of acquiring skin cancer. Biologically, this is supported by previous studies that have shown that UV radiation induces both DNA damage and vitamin D synthesis (Holick *et al.*, 1977, 1979; Adams *et al.*, 1982; MacLaughlin *et al.*, 1982; Freeman *et al.*, 1989), that vitamin D formation in the skin increases with increasing UV-B radiation exposure (Adams *et al.*, 1982; MacLaughlin *et al.*, 1982), and that in areas with high UV index the incidence of non-melanoma skin cancer and, to a lesser degree, melanoma is increased in Caucasians (Armstrong and Kricker, 2001), as are plasma 25-OH-vitD levels (McCullough *et al.*, 2010).

In support of our findings that elevated plasma 25-OH-vitD may be used as a risk factor for non-melanoma skin cancer and melanoma, a previous cohort study demonstrated that elevated plasma 25-OH-vitD levels associate with increased risk of non-melanoma skin cancer (Asgari *et al.*, 2010). However, other case–control and cohort studies have shown an inverse relationship between plasma 25-OH-vitD and risk of non-melanoma and melanoma skin cancer (Tang *et al.*, 2010; Newton-Bishop *et al.*, 2011). Contrary to the latter studies, a randomized study did not show the expected decreased risk of non-melanoma and melanoma skin cancer with vitamin D supplementation (Tang *et al.*, 2011a), although a *post hoc* analysis did show that supplementing with vitamin D and calcium reduced the risk of melanoma in cases with a history of non-melanoma skin cancer. However, the authors themselves were not confident that this result was genuine. Compared with previous studies on the association of plasma 25-OH-vitD and risk of non-melanoma and melanoma skin cancer, our study has more cases of non-melanoma skin cancer, although fewer cases of melanoma, a longer follow-up, and a more complete case ascertainment due to the Danish Cancer Registry.

A potential limitation is that our cohort consists of whites of Danish descent living in Denmark (55°–58° North) with less sun exposure than closer to the equator; consequently, our

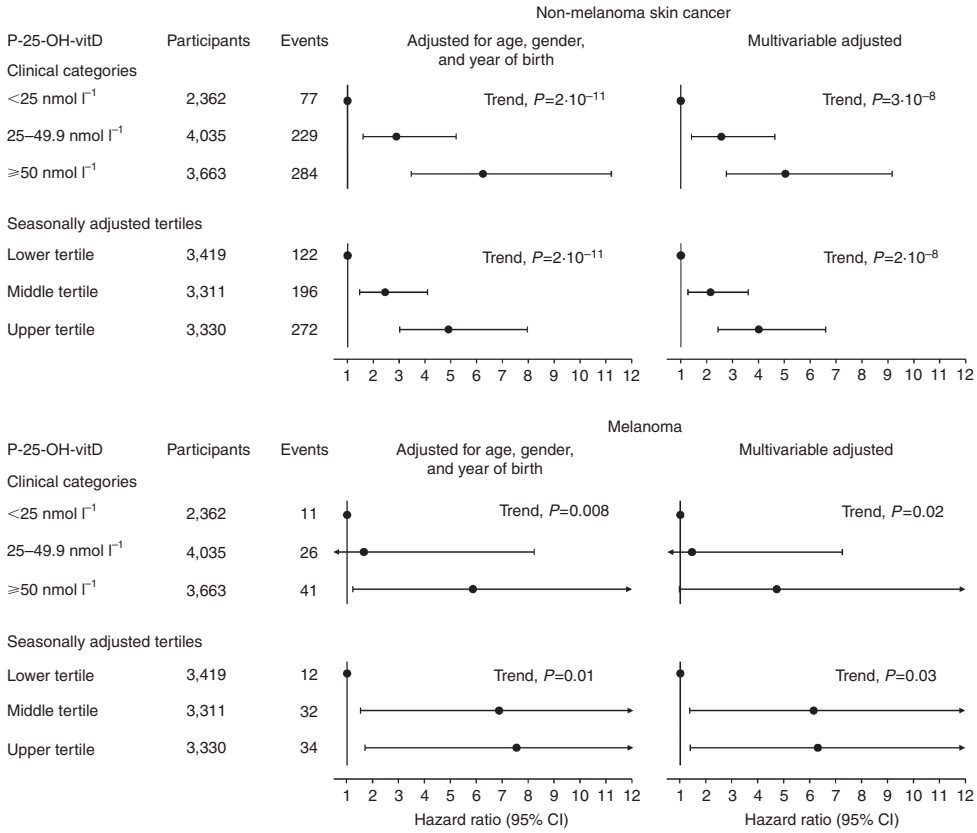

Figure 4. Hazard ratios for non-melanoma skin cancer and melanoma skin cancer by plasma 25-hydroxyvitamin D (P-25-OH-vitD). Hazard ratios for non-melanoma skin cancer and melanoma skin cancer by P-25-OH-vitD in clinical categories and in seasonally adjusted tertiles (expressed as percentiles). Multivariable models were adjusted for gender, pack-years, body mass index, income, occupational physical exertion, intensity of leisure-time activities, and regular cycling or running. Furthermore, the models using the clinical categories for 25-OH-vitD were adjusted for month of blood sample. The analyses are adjusted for age and year of birth to account for calendar effects. Risk estimates were corrected for regression dilution bias (based on 10,060 individuals from the Danish general population, the Copenhagen City Heart Study, followed for up to 28 years after blood sampling).

findings would be most relevant for individuals with a similar level of sun exposure and for similar 25-OH-vitD levels. Furthermore, skin phenotype or pigmentation was not recorded. Similarly, sun exposure and sunburns during holidays in Denmark or abroad were not recorded, and as sunburn is predictive of melanoma risk this represents another limitation. A sun-exposure variable that takes into account incidence of sunburn and sun exposure would have been preferable, especially considering that one of our sun-exposure variables, occupational physical exertion, was not associated with vitamin D levels. The delay in measurement from blood sampling in 1981–1983 to measurement in 2009–2010 could raise concern of potential decay of plasma 25-OH-vitD. However, we observed the expected seasonal variation in plasma 25-OH-vitD, that the median levels across plasma samples with storage times of 10, 20, and 30 years were similar, the median levels were comparable to similar populations (McCullough *et al.*, 2010; Durup *et al.*, 2012; Husemoen *et al.*, 2012), 25-OH-vitD measurements are very resistant to preanalytical conditions and storage times of several years (Lissner *et al.*, 1981; Ocke *et al.*, 1995; Antoniucci *et al.*, 2005), and a reduced quality of plasma 25-OH-vitD measurements would tend to weaken associations rather than inflate them. This potential limitation thus cannot explain our positive results, and in fact the well-preserved seasonal variation in plasma 25-OH-vitD with the highest levels in September and the lowest levels in February suggest that baseline 25-OH-vitD is a valid marker of sun exposure of the individual. Despite the generally high coverage of the Danish Cancer Registry (Storm, 1988, 1991; Storm *et al.*, 1997), we cannot exclude underreporting of non-melanoma skin cancers, as they are difficult to register (owing to lack of histological verification, treatment in private settings, etc), and extra checks might have been needed to ensure that all non-cases were free of non-melanoma skin cancer, which was not done. However, the admixture of cases into the non-case population would tend to weaken the association rather than inflate it, and thus cannot explain our results.

The strengths of our study are the up to 28 years of complete follow-up, a homogenous population, and high case ascertainment. We used a random sample of the general population, the Copenhagen City Heart Study, which makes our results applicable to the general population. In addition, we measured plasma 25-OH-vitD that reflects internal vitamin D status, which is superior to evaluating dietary intake alone. Moreover, the association of plasma 25-OH-vitD with risk of

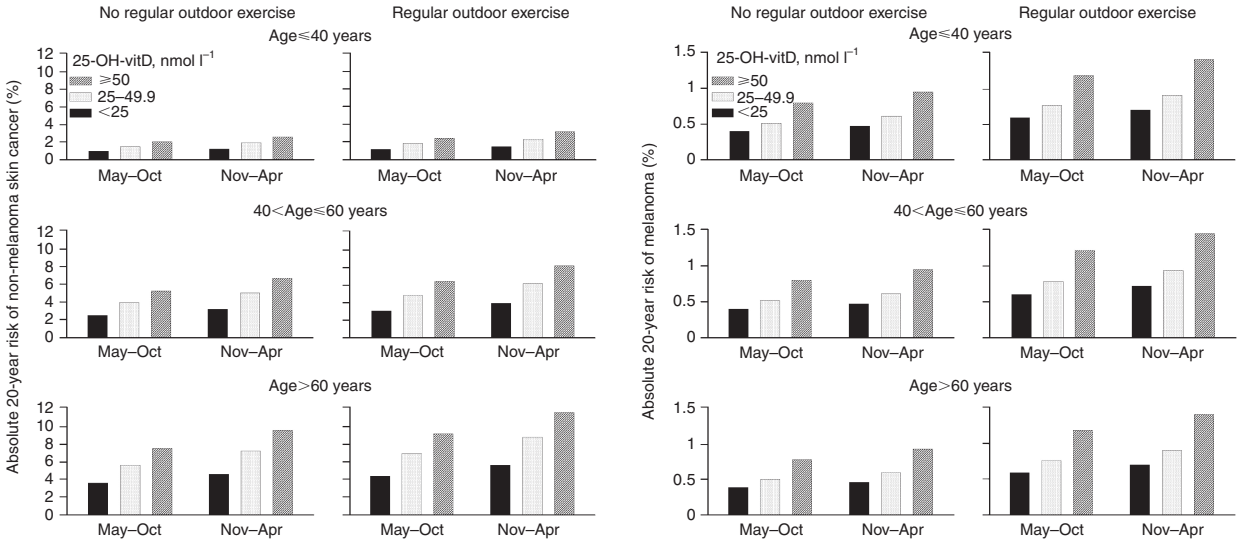

Figure 5. Absolute 20-year risk of non-melanoma skin cancer and melanoma skin cancer. Absolute 20-year risk of non-melanoma skin cancer and melanoma skin cancer by 25-hydroxyvitamin D levels, age, season of blood sampling, and participation in regular outdoor exercise (running or cycling) calculated by using the regression coefficients from a Poisson regression model. Apr, April; Nov, November; Oct, October.

non-melanoma skin cancer and melanoma was robust irrespective of the method of seasonal adjustment and statistical model, i.e., the association was present in relative risk and absolute risk models, as well as in differently adjusted models. Finally, because we measured 25-OH-vitD on 400 individuals 10 years apart, we were able to correct for regression dilution bias, i.e., a correction that helps avoid underestimation of risk estimates owing to imprecise measurement of 25-OH-vitD (Clarke *et al.*, 1999).

In Northern Europe, UV-B radiation from the sun is only adequate for sufficient endogenous vitamin D production in the skin during the summer months, and food has never been fortified with vitamin D in Denmark. The present study therefore allows for the determination of the natural history of the association of vitamin D levels with risk of non-melanoma and melanoma skin cancer. However, through dietary consumption of vitamin D–rich products and through the use of supplements, plasma 25-OH-vitD levels might increase, most likely without increasing the risk of skin cancer. As in recent years much more attention is being paid to vitamin D, plasma levels measured in the early 1980s are likely less influenced by such supplements than would be currently the case. Thus, in populations deriving the majority of their vitamin D from dietary sources, the present observed association may be attenuated, either in the current cohort if the participants changed their behavior as indicated during the follow-up period or in more recent cohorts having been collected after the focus on vitamin D supplementation has increased.

Our study adds to the risk–benefit discussion regarding sun exposure in two important ways: first, that plasma 25-OH-vitD probably acts as a surrogate marker for sun exposure at the population level and, second, that there is an increase in skin cancer risk when going from the low range of 25-OH-vitD to the high range, i.e., recommended sun exposure may be enough to induce premalignant or malignant changes in the skin. However, our study cannot, and is not designed to, assess the risk–benefit profile of sun exposure or increased 25-OH-vitD with regard to other health outcomes, which could justify current recommendations regarding daily sun exposure. Similarly, our study does not definitely disprove the hypothesis that levels in the sufficient range of plasma 25-OH-vitD can reduce the risk of developing skin cancer.

In conclusion, we have shown that increasing levels of plasma 25-OH-vitD are associated with increased risk of non-melanoma skin cancer and melanoma.

MATERIALS AND METHODS

Study design

The Copenhagen City Heart Study is a prospective cohort study of the Danish general population initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1994, and 2001–2003 (Bojesen *et al.*, 2003; Nordestgaard *et al.*, 2007). Individuals aged 20–100 years were drawn randomly from the national Danish Central Person Register to reflect age and gender distribution in the whole population and invited to participate; all inhabitants in Denmark are uniquely identified through their central person registration number that also holds information on date of birth and gender.

The present study included 10,060 participants from the 1981–1983 examination: 17,312 were invited, 12,214 participated (71%), and 10,060 (58%) had available plasma samples for 25-OH-vitD measurement; 147 participants with a diagnosis of skin cancer before study entry were excluded (see Cancer end points).

A Danish ethics committee approved the study (KF100.2039/91 and KF01-144/01). The Declaration of Helsinki Principles were followed and all participants provided written informed consent.

Measurements of 25-OH-vitD

Plasma samples collected at baseline in 1981–1983 were stored at −20°C until 2009–2010 when 25-OH-vitD was measured using the DiaSorin Liaison 25-OH-vitD TOTAL assay (Ersfeld *et al.*, 2004). Assay precision was tested daily, whereas assay accuracy was tested

monthly using an external quality-control program. The inter-assay coefficient of variance was 10% for low-level controls ($\sim$40 nmol l^{-1}) and 8% for high-level controls ($\sim$135 nmol l^{-1}).

Covariates

Selection of covariates was based on review of literature on determinants of skin cancer risk and levels of vitamin D (Rubin *et al.*, 2005; Asgari *et al.*, 2009; McCullough *et al.*, 2010; Pothiawala *et al.*, 2012). Variables suspected to possibly affect both skin cancer risk and vitamin D levels were selected as covariates.

Information on smoking habits was obtained from self-reported questionnaires completed together with an examiner on the day of attendance. Daily tobacco consumption (grams per day) was calculated for current smokers. Cumulative tobacco consumption was calculated for former and current smokers in pack-years; a pack-year was defined as 20 g of tobacco per day for a year. BMI was calculated as measured weight (kilograms) divided by measured height (meters) squared. Participants were also asked about their level of income. Information on occupational physical exertion, intensity of leisure-time activities, and running and cycling habits were obtained from self-reported questionnaires and used as surrogate markers for sun exposure; i.e., all three variables were included in our analyses as separate variables to capture sun exposure.

Cancer end points

Diagnoses of non-melanoma and melanoma skin cancer from 1943 to December 2008 were obtained from the Danish Cancer Registry that identifies 98% of cancer cases in Denmark from all hospitals and private practicing pathologists (Storm, 1988, 1991; Storm *et al.*, 1997). This is required by law. The diagnoses from date of blood sampling and onward were classified according to the World Health Organization international classification of diseases 10th edition (ICD-10): non-melanoma skin cancer as C44 and melanoma as C43. None of the non-melanoma skin cancer or melanoma diagnoses were based on self-report, and all were from the Danish Cancer Registry. Both cancer types were recorded from 1943. In addition, from 1978 and onward histological subtypes of non-melanoma skin cancer, i.e., basal cell carcinoma, spinocellular carcinoma, and other rare histological types of non-melanoma skin cancer, were all recorded.

Follow-up time for each subject began at the day of blood sampling in 1981–1983 and ended at first skin cancer, death ($n=6{,}268$), emigration ($n=54$), or December 2008, whichever occurred first. The median follow-up time to first skin cancer, death, emigration, or December 2008 was 20.5 years (range 0.04–28). Follow-up was 100% complete, i.e., we did not lose track of a single individual.

Statistical analyses

A priori we divided baseline plasma 25-OH-vitD into the following clinical categories of >50 nmol l^{-1} (sufficient levels), 25–50 nmol l^{-1} (insufficient levels), and <25 nmol l^{-1} (deficient levels). In addition, because concentration of 25-OH-vitD was expected to vary according to time of the year owing to the high-latitude geographical position of Denmark, we also used seasonally adjusted 25-OH-vitD levels. Two strategies were applied to adjust for the seasonal variation in vitamin D. First, we used unadjusted 25-OH-vitD levels in regression analyses while adjusting for calendar month of blood sampling. Thus, 25-OH-vitD was adjusted for calendar month of blood sampling when using clinical categories in analyses. Second, calendar month–specific cutoff points were obtained by assigning subjects to percentile categories within the same month of sampling. We divided the seasonally adjusted values into tertiles. For trend tests across ordered groups, individuals were assigned the median value of their group, either as absolute values or as percentiles.

Cumulative incidences were estimated using the competing risk proportional subhazard models by the method of Fine and Gray (Fine and Gray, 1999), in which competing risk of death was accounted for. The analyses were adjusted for age and year of birth to account for calendar effects. We used age as the time scale. The cumulative incidence functions were plotted by seasonally unadjusted clinical categories and seasonally adjusted percentile categories.

Cox proportional hazards regression was used to estimate hazard ratios with 95% CI for incident cancer. We used age as the time scale with delayed entry (left truncation). Thus, age differences were automatically adjusted for and referred to in text, tables, and figures as age adjusted. However, for the test of interaction of age with 25-OH-vitD levels on cancer risk, we used years of follow-up as the time scale, as using age as the time scale does not allow testing for interactions with age. Furthermore, all analyses were adjusted for year of birth, in addition to age, to account for calendar effects. Multivariable adjusted Cox regression models included gender, age, cumulated tobacco consumption in pack-years, BMI, income, occupational physical exertion, physical intensity of leisure-time activities, running and cycling habits, and calendar month of blood draw. Interactions were tested for using likelihood ratio tests with Cox regression models including and excluding multiplicative two-factor interaction terms. The proportional hazards assumption was tested for in Cox regression models using Schoenfeld residuals; no departures were detected for the different plasma 25-OH-vitD variables used. Missing data were imputed using multivariable chained imputation (STATA:mi impute chained), in which age and gender were independent variables and BMI, physical intensity of leisure-time activities, and income were dependent variables in the model. Hazard ratios and CIs were corrected for regression dilution bias (Clarke *et al.*, 1999), using plasma 25-OH-vitD from 400 individuals without non-melanoma skin cancer, melanoma skin cancer, other cancers, or other chronic diseases participating in both the 1991–1994 and 2001–2003 examinations of the Copenhagen City Heart Study; this correction helps to avoid underestimation of risk estimates, but does not affect whether results are significant. The regression dilution ratio was 3.1 on samples taken 10 years apart and 2.2 on samples taken 20 years apart; the latter was used to adjust risk estimates.

Estimated absolute risks for non-melanoma and melanoma skin cancer were calculated by using the regression coefficients from a Poisson regression model with the same covariates as the Cox regression and, in addition, age at entry in three groups ($\leqslant$40 years, $>$40 to $\leqslant$60 years, and $>$60 years). We analyzed the data with the statistical package STATA 11.2 (STATA, College Station, TX).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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